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Preservation of lymphopoietic potential and virus suppressive capacity by CD8⁺ T-cells in HIV-2 infected controllers

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Running title: Effective CD8+ T cells in HIV-2 infected controllers

Abstract

Compared to Human immunodeficiency virus 1 (HIV-1), HIV-2 infection is characterized by a larger proportion of slow or non-progressors. A better understanding of HIV-2 pathogenesis should open new therapeutic avenues to establish control of HIV-1 replication in infected patients. Here, we studied the production of CD8⁺ T cells and their capacity for viral control in HIV-2 controllers from the French ANRS CO5 HIV-2 cohort. HIV-2 controllers display a robust capacity to support long-term renewal of the CD8⁺ T-cell compartment by preserving immune resources, including hematopoietic progenitors and thymic activity, which could contribute to the long-term maintenance of the CD8⁺ T-cell response and the avoidance of premature immune aging. Our data support the presence of HIV-2 Gag-specific CD8⁺ T-cells that display an early memory differentiation phenotype and robust effector potential in HIV-2 controllers. Accordingly, we show for the first time that HIV-2 controllers possess CD8⁺ T-cells that show an unusually strong capacity to suppress HIV-2 infection in autologous CD4⁺ T- cells *ex vivo*, an ability that likely depends on the preservation of host immune resources. This effective and durable antiviral response probably participates in a virtuous circle, during which controlled viral replication permits the preservation of potent immune functions, thus preventing HIV-2 disease progression.

Introduction

In the absence of an effective vaccine that prevents the HIV-1 acquisition, the long-term suppression of viral replication in infected individuals remains a major objective to prevent further disease progression and dissemination. Although progression to AIDS can now be significantly delayed by effective combinatorial antiretroviral therapy (cART), HIV remission, which is observed in HIV-1 controllers (HIC1) (1) and rare patients who gain independence from ART after early initiation (2), remains a priority. Much effort, therefore, has been given to the identification of immune correlates of natural HIV control in an attempt to decipher the immunological mechanisms that underlie effective control of HIV-1 replication in the absence of ART. A better understanding of these correlates would provide insights into therapeutic approaches that would modulate the immune system to mimic the immune parameters of HIV-1 controllers, ultimately leading to a functional cure of HIV-1 infection.

Although HIV-2 shares the same modes of transmission and intracellular mechanisms of replication as HIV-1, it provides a unique model of attenuated infection by a human immunodeficiency virus. Compared to HIV-1 infection, HIV-2 infection is predominantly characterized by a slower decline of CD4⁺ T-cells and lower levels of immune activation.(1-3). Whilst HIV-2 infected patients who progress towards disease present similar clinical manifestations and AIDS severity as those infected with HIV-1, they remain a minority (~15-20% of those infected (4)). Many HIV-2 infected individuals spontaneously control their infection and remain asymptomatic while maintaining undetectable viral loads, signifying a high prevalence of HIV-2 controllers (HIC2) (9.1% of HIC2 in the ANRS CO5 HIV-2 cohort (5) vs 0.22% HIC1 in the ANRS CO4 FHDH (French Hospital Database on HIV)(6)). Deciphering the

immune correlates of HIV-2 control and contrasting these findings with those from HIV-1 controllers may illuminate the key features of HIV pathogenesis and provide critical information on what is needed to establish natural control of HIV.

It is well established that CD8⁺ T-cell mediated immunity is critical in the control of HIV-1 replication from the earliest days of acute HIV-1 infection. However, the HIV-specific T-cell response declines over time and is often lost in the later phases of chronic infection (7). Moreover, HIV-1 infected individuals with protracted infection usually demonstrate reduced thymic output and lower naïve T-cell numbers (8-10). This situation, which is reminiscent of immune aging, likely accounts for the loss of CD8⁺ T-cell regenerative capacity over time, and the failure of the T-cell response to adapt to the emergence of escape variants. Only rare HIC1 (often possessing specific “protective” HLA-class I alleles (11)), are able to maintain viremia spontaneously and durably at extremely low levels (12). Furthermore, this level of control has been associated with their capacity to develop and sustain the production of HIV-specific CD8⁺ T-cells that are endowed with the capacity to efficiently suppress HIV-1 infection of autologous CD4⁺ T-cells (13).

In the present work, we focus on the potential role of cellular immunity as a key player in controlling HIV-2 infection. Strong Gag-specific CD8⁺ T-cell responses have been previously observed in HIV-2 infected patients, and their magnitude was inversely associated with viremia (14, 15). Of note, these HIV-2 Gag-specific CD8⁺ T-cells were described as polyfunctional, although they did not contain high levels of cytolytic markers (16). Persistent CD8⁺ T-cell renewal is therefore likely to be essential for maintaining long term immune efficacy against HIV. We embarked here on the fine characterization of HIV-2-specific CD8⁺ T-cells in HIC2 from the French ANRS CO5 HIV-2 cohort, with particular focus on the

98 production of HIV-specific CD8⁺ T-cells and their HIV-2 suppressive capacity. We describe for
99 the first time that CD8⁺ T-cells from HIC2 demonstrate strong capacity to suppress HIV-2
100 infection in autologous CD4⁺ T-cells *ex vivo*. Importantly, HIC2 retain robust lymphopoiesis in
101 general and CD8⁺ T-cell production in particular, suggesting that the marked premature
102 immune aging phenotype seen in HIV-1 infection is not present in these individuals, which is
103 likely to be key to the maintenance of a durable and efficient CD8⁺ T-cell response. Overall,
104 our data suggest that the immune correlates of effective control in HIV-2 infected individuals
105 may derive from the capacity of the immune system to maintain the potent HIV suppression
106 capacity that is mediated by CD8⁺ T-cells.

107

Material and Methods

Study subjects

HIV-2 controllers (HIC2) were part of the ANRS CO5 VIH-2 cohort and included in the ANRS IMMUNOVIR 2 study, which focuses on the study of patients with non-progressive infection. All patients in the present study had characteristic features of HIV controllers i.e. asymptomatic treatment naïve individuals, infected for at least 5 years, with a CD4⁺ T cell count > 400 cells/μl and a viral load < 400 RNA copies/ml.

HIC2 were compared to HIV-1-infected individuals: HIV-1 controllers (HIC1) from the ANRS CO21 Codex cohort and two groups of HIV-1 viremic individuals (one with CD4⁺ T cell count below 200 cells/ml and another with CD4⁺ T cell count above 500 cells/ml) from the Pitié Salpêtrière Hospital (France). Mononuclear cells were isolated over a Lymphoprep gradient and then either used directly or cryopreserved. PBMC from uninfected healthy adults from the French Blood Bank (Etablissement Français du Sang) were also analyzed. A summary of clinical attributes of the patients studied is displayed in Table II.

All participants gave their written informed consent. The study was approved by the institutional ethics committee (i.e. Comité de Protection des Personnes of Ile de France XI).

Flow cytometry and reagents

In order to generate tetramers, immunodominant HIV-2 epitopes were identified by screening HIV-2 p27 overlapping peptides in IFNγ elispot assays using HLA typed HIV-2 infected patient PBMCs, going on to define the optimal epitope sequences and length (15).

HLA-B*5301 p27 TPYDINQML (TL9) and p27 DRFYKSLRA (DA9) tetramers were synthesized as previously described (17). PBMC were stained with pre-titrated concentrations of pentamer/tetramer (conjugated to PE) followed by a panel of antibodies as previously described (18). Directly conjugated and unconjugated antibodies were obtained from the following vendors: BD Biosciences (BD Biosciences): CD34 (PE), lineage cocktail (CD3, CD14, CD16, CD19, CD20, CD56 / FITC), CD3 (PerCP-Cyanine 5.5] or Alexa Fluor 700), CD45RA (FITC), CCR7 (PE-Cyanine 7), CD107 α (PE-Cyanine 5), IFN γ (Alexa Fluor 700), and TNF α (PE-Cyanine 7); Beckman Coulter (Beckman Coulter): CD28 (PE-TexasRed), CD45RA (PE-TexasRed); Caltag (Thermo Fisher): CD8 (Alexa Fluor 405), granzyme B (PE-TexasRed); R&D Systems (R&D Systems Europe): MIP-1 β (FITC); BioLegend (Ozyme): CD127 (BV-650), CD27 (Alexa Fluor 700). Cell surface marker stainings were performed using standard methodologies. CD34⁺ cell phenotyping was performed on enriched populations. The immunomagnetic enrichment of CD34⁺ cells was carried out on PBMCs using MACS technology, according to the provider's recommendations (Miltenyi Biotech). Stainings were analyzed on an LSR Fortessa flow cytometer (BD Biosciences) and data analyzed using FlowJo v8.2 (FlowJo, LLC) and DIVA (BD Biosciences) softwares.

Intracellular cytokine staining assay

Purified PBMC were thawed and rested overnight at 37°C in complete RPMI media (RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, L-glutamine and antibiotics); viability was then examined by trypan blue exclusion (typically \geq 70% viable). For stimulation, cells were then incubated in the presence of 15-mer overlapping peptides

covering the HIV-2 p27 protein (10 μ M) or an overlapping peptide pool encompassing clade B HIV-1 Gag (2 μ g/ml). p27 stimulated cells were incubated in the presence of anti-CD107 α antibodies for 1h at 37 °C in a 5% CO₂ incubator, followed by an additional 5h in the presence of the secretion inhibitors monensin (2.5 μ g/mL; Sigma-Aldrich) and Brefeldin A (5 μ g/mL; Sigma-Aldrich). BD Cytofix/Cytoperm™ (BD Biosciences) was used for permeabilization of the cells prior to staining for intracellular markers.

***In vitro* HIV-2 suppression assay**

To assess the capacity of CD8⁺ T-cells to suppress HIV-2 infection of autologous CD4⁺ T-cells *in vitro*, we adapted our previously published HIV-1 suppression assay (19). Briefly, PBMC were isolated from peripheral blood by density centrifugation. CD4⁺ and CD8⁺ T-cells were then isolated by, respectively, positive and negative magnetic-bead sorting (STEMCELL Technologies). CD4⁺ T-cells were activated in complete RPMI media supplemented with phytohemagglutinin (1 μ g/ml) and interleukin-2 (IL-2) (100 IU/ml) for 3 days. CD8⁺ T-cells were cultured in non-supplemented culture media. Activated CD4⁺ T-cells were infected with HIV-2 SBL (20) using a spinoculation protocol (21) and cultured alone or with autologous CD8⁺ T-cells at a 1:1 ratio during 14 days in IL-2 (100 IU/ml) supplemented culture media. Viral replication was measured by p27 production in culture supernatants every 3-4 days as determined by enzyme-linked immunosorbent assay (Gentaur). The capacity of CD8⁺ T-cells to suppress HIV infection was calculated at the peak of viral replication as the log decrease in p27 production when superinfected CD4⁺ T-cells were cultured in the presence of CD8⁺ T-cells ($\log[\text{p27 in CD4 T-cell culture}/\text{p27 in CD8:CD4 1:1 co-cultures}]$).

173

174 **TREC quantification**

175 Thymic function was estimated by quantification of signal joint T-cell reception excision
176 circles (sjTREC) as previously described (22). Briefly, PBMC lysates were subjected to
177 multiplex polymerase chain reaction (PCR) for 22 cycles using sjTREC and CD3 γ chain outer
178 primer pairs. Each of the amplicons was then quantified using Light Cycler technology (Roche
179 Diagnostics), performed on 1/100th of the initial PCR, in independent experiments, but on
180 the same first-round, serially diluted standard curve. This highly sensitive, nested
181 quantitative PCR assay allows detecting one copy of sjTREC in 10⁵ cells. The sjTRECs were
182 quantified in triplicate.

183

184 **Statistical analysis**

185 Statistical analyses were performed using Prism software (GraphPad). Non-parametric tests
186 of significance were performed throughout all analyses, using Kruskal-Wallis and Mann-
187 Whitney testing for intergroup comparisons and Spearman rank test to determine
188 correlations. *P* values >0.05 were considered not significant.

189

Results

HIV-2-positive population characteristics

We studied 20 HIV-2-positive individuals with controlled viremia in the absence of cART. The detailed characteristics of the patients are reported in Table I. The proportion of female patients was 60% (n=12); the median age was 49 (Interquartile range (IQR): 42 - 52). At inclusion, the median time since HIV-2 infection diagnosis was 13.6 years (IQR: 9.7 - 19.3) and the median CD4⁺ T-cell counts were 893 cells/ μ l of blood (IQR: 707 - 1170 cells/ μ l). Two individuals had detectable, albeit low, HIV-2 viral load (54 and 117 RNA copies/ml). Three individuals were born in France, 1 in Colombia and 16 in west-African countries (Table I).

Only two of the HIV-2 infected patients studied carried the "HIV-1 protective" B*57 allele and just one the B*27 allele. The most common class I HLA allele was C*04, which was carried by nine individuals (45%), of whom five were homozygotes (Table I). All five C*04/C*04 individuals were born in Ivory Coast. C*04 is a relatively common gene in France and West Africa. Overall, 11 (55%) individuals expressed homozygous alleles, suggesting that these individuals came from bottleneck populations with limited genetic diversity.

HIV-2 controllers show preserved CD8⁺ T-cell production capacities

We previously showed that exhaustion of lymphopoiesis is a major correlate of disease progression in HIV-1 infection (9, 23). Our analyses highlighted a marked decline of primary immune resources in HIV-1 progressors, including CD34⁺ hematopoietic progenitor cells (HPC) and naïve CD4⁺ and CD8⁺ T-cell numbers. These alterations, which are the

hallmark features of advanced immune aging, were not observed in HIC1. We therefore performed similar analyses in HIC2, directly analyzing the frequency and phenotype of CD34⁺ hematopoietic progenitor cells (Fig. 1A, 1B), as well as the frequency of naïve CD8⁺ T-cells from blood (Fig. 1C). The frequency of circulating HPCs (CD34⁺Lin⁻) in HIC2 was high compared to levels found in untreated HIV-1 infected patients with low CD4⁺ T-cell counts, and equivalent to those observed in HIC1 (Fig. 1A). In a similar manner, HIC2 displayed a high proportion of lymphoid precursor progenitor cells that were identified by the CD117⁺ CD45RA⁺ phenotype among CD34⁺ cells (Fig. 1B). Moreover, we found high levels of naïve lymphocytes within the CD8⁺ T-cell compartment of these individuals (Fig. 1C). Our results demonstrate the maintenance of effective lymphopoiesis in HIV-2 infection, which is likely to be linked with a capacity to sustain the long-term production of naïve CD8⁺ T-cells.

In HIV-1 infection, the progressive decrease in naïve T-cell proportions in HIV-infected adults likely results from the antigen-driven maturation of these cells in the absence of adequate T-cell renewal capacity due to the declining thymic output of new cells (8, 10, 24). We and others previously reported that thymic function is preserved in HIV-2-infected individuals with low viral load (25). It was also recently shown that HIV-2 infection in thymocytes is impaired (26, 27), which may contribute to the maintenance of high CD4⁺ T-cell counts in HIV-2 infected patients. Here, we measured signal joint T-cell receptor (sjTCR) excision circles (TREC) levels in total PBMCs to estimate thymic function in HIC2. Naïve CD8⁺ T-cell counts directly correlated with the amount of sjTREC/ml (Fig. 1D), suggesting that the maintenance of these cells was related to sustained thymic activity. This is in line with the correlation between naïve CD8⁺ and naïve CD4⁺ T-cell counts in blood taken from the same individuals (Supplementary Fig. 1).

In addition, we assessed the levels of recent thymic emigrants using CD31 expression (28), which primarily provides information on the thymic history of naïve CD4⁺ T-cells. In line with the TREC data, a strong correlation was observed between the frequency of recent thymic emigrants (i.e. CD31⁺ naïve CD4⁺ T-cells) and the naïve CD8⁺ and CD4⁺ T-cell counts (Fig. 1E and Supplementary Fig. 1). Taken together, these data support the maintenance of a robust lymphopoietic capacity and thymic output in HIC2 and signifies robust preservation of the naïve CD8⁺ T-cell compartment. The latter is central to the mounting of *de novo* responses, as recently shown in elderly subjects (29) and old vaccinated primates (30), and the ability to sustain effective T-cell responses against a virus with high evolutionary rates, as is the case with HIV.

HIV-2 Gag-specific CD8⁺ T-cells display early memory differentiation and robust effector potential

We next performed a comprehensive characterization of HIV-2-specific CD8⁺ T-cell responses. Since the IFN γ response of Gag-specific CD8⁺ T-cells was found to correlate inversely with viremia in HIV-2 infected individuals, suggesting an important role of these cells in controlling HIV-2 infection (15), we decided to focus on phenotyping CD8⁺ T-cells that were specific for p27, which is the major component of HIV-2 Gag. To assess the overall magnitude of HIV-2 Gag-specific CD8⁺ T-cells in our patients, we performed intracellular IFN γ staining on CD8⁺ T-cells from PBMCs that were stimulated with 15 mer overlapping peptides that spanned the proteome of the HIV-2 p27 protein. Robust p27-specific CD8⁺ T-cell responses were detected in most patients (Fig. 2A). These responses were of similar

magnitude to those observed in HIC1, although a direct comparison cannot be made, considering that a different antigen would be used to stimulate HIV-1-specific responses. These cells also exhibited other effector functions, as revealed by co-staining for TNF α , MIP-1 β and CD107 α (Supplementary Fig. 2), highlighting a polyfunctional profile that has been previously described (31). We furthered our investigation by using recombinant MHC class I-peptide tetrameric complexes, which more precisely capture the memory differentiation phenotype of p27-specific CD8⁺ T-cells (Fig. 2B). We analyzed in particular CD8⁺ T-cells that were restricted by HLA-B*5301, since B*53 was the most common allele in our group of patients. We were able to generate HLA-B*5301 tetramers with the immunodominant p27 epitope TPYDINQML (TL9). All of the seven HLA-B*53 patients tested positive for TL9-specific CD8⁺ T-cells. We also detected HLA-B*1401 restricted p27 DA9 (DRFYKSLRA)-specific CD8⁺ T-cells in two out of two HLA-B*14 patients.

The differentiation phenotype of HIV-2-specific CD8⁺ T-cells was assessed based on the surface expression of the receptors CD27, CD28, CCR7, CD45RA and CD127 (Fig. 2C), and compared to the phenotype of HLA-B*2705 restricted p24 KK10-specific CD8⁺ T-cells, the latter selected as the prototype of primary effector cells in HIV-1 infection (18, 32). Overall, both groups of HIV-specific CD8⁺ T-cells displayed similar phenotypes, being mainly CD27⁺ CCR7⁻ CD127⁻, with slight but noticeable differences. For example, HIV-2-specific CD8⁺ T-cells trended towards higher CD45RA expression. In HIV-1 infected patients, CD45RA expression was the hallmark of a rare population of resting cells that resemble long-lived memory cells and were particularly noticeable shortly after viral replication was curtailed by cART (33). Furthermore, the expression of CD28 was significantly higher on HIV-2-specific CD8⁺ T-cells (Fig. 2D), in line with previous observations made with HLA-B*3501 restricted p27

NPVPVGNIY (NY9)-specific CD8⁺ T-cells (34). This indicates that HIV-2-specific CD8⁺ T-cells are in general less differentiated (35, 36), displaying an even younger phenotype than highly effective HIV-1-specific CD8⁺ T-cells. Accordingly, HIV-2 specific CD8⁺ T-cells are likely to retain proliferative potential, as previously suggested (34). Nevertheless, despite their early memory differentiation state, HIV-2-specific CD8⁺ T-cells express high levels of the transcription factors T-BET and EOMES (Supplementary Fig. 3), which are key regulators of memory differentiation and the acquisition of effector functions (37). In accordance with the expression of these transcription factors, HIV-2-specific CD8⁺ T-cells possessed high intracellular levels of granzyme B, at least relative to what is seen in effective HIV-1-specific CD8⁺ T-cells (Fig. 2D). Taken together, these data support the presence of robust Gag-specific CD8⁺ T-cell responses in HIC2. These cells are characterized by an early or young differentiation phenotype and display strong effector potential.

Unstimulated CD8⁺ T-cells from HIV-2 controllers suppress HIV-2 infection *ex vivo*

Lastly, we aimed to show that this strong phenotypic functional potential translated into an effective capacity of these cells to suppress HIV-2 infection. We previously showed that HIC1 often possess HIV-specific CD8⁺ T-cells with a striking capacity to suppress infection by killing HIV-infected autologous CD4⁺ T-cells (38, 39). This capacity was not found in cART treated or untreated viremic HIV-1 patients, either during the acute or chronic phase of infection (38-41). HIV-1 suppression assays provide the most robust method to distinguish between the functional capacities of CD8⁺ T-cells from HIV-1 controllers and non-controllers

(40, 42, 43). We thus sought to adapt this *in vitro* assay to the study of HIV-2 using an SBL virus strain (20), with supernatant p27 concentration as a read-out.

We found that CD8⁺ T-cells from HIC2 have a strong capacity *ex vivo* to suppress HIV-2 infection of autologous CD4⁺ T-cells (Fig. 3A), with a median reduction in p27 production of 2.7 logs [IQR: 1.3-3.3] (Fig. 3B). The viral suppressive capacity shown by the CD8⁺ T-cells from HIC2 was remarkably high, comparable to the levels observed with cells from HIC1 from the ANRS CO21 cohort (Fig. 3B) or even among our previous studies (38, 39) . Similarly to what we found in HIV-1 controllers (39), the capacity of CD8⁺ T-cells from HIV-2 controllers to suppress infection was relatively stable over time (Fig. 3C). There was however some heterogeneity in HIV-2 suppressive capacity among patients, with logp27 decrease values ranging from 0.02 to 3.7 (Fig. 3B). Interestingly, in two patients who had the steepest decline in CD4⁺ T-cell counts over the 3 years preceding study inclusion (92.5 and 215.7 cells/mm³/year), their CD8⁺ T-cells showed no *ex vivo* capacity to suppress HIV-2 infection in our assay (Fig. 4A). Accordingly, we found a positive correlation between capacity of CD8⁺ T-cells to mediate HIV-2 suppression *ex vivo* and the patients' CD4 T-cell counts (Fig. 4B), which supports the key role of these CD8⁺ T-cells in suppressing HIV-2 infection and preventing disease progression. These results are in line with previous studies showing that strong CD8⁺ T-cell mediated HIV-1 suppression *ex vivo* correlates with better clinical outcome (38, 44, 45), and strengthen the value of the HIV-1/2 suppression assay as a correlate of HIV control.

Discussion

Although the immune dynamics responsible for the slow progression of HIV-2 infection has remained puzzling for years, we are gaining momentum in piecing together the precise picture of events that are responsible for this largely non-progressive HIV infection. At equivalent plasma viral loads, proviral DNA loads are very similar between HIV-1 and HIV-2 infections (1, 46-48). When left unchecked by cART, the survival of HIV-2 infected patients is strongly related to both CD4⁺ T-cell counts and plasma viral load. However, in contrast to HIV-1, HIV-2 is often controlled to undetectable plasma viral loads in the absence of cART, which most likely involves a complex immune-related mechanism.

Owing to the maintenance of a strong lymphopoietic capacity, T-cell mediated immune responses appear to be better preserved in HIV-2 infection and HIC2 can sustain the robust suppression of viremia over several years or even decades of chronic infection. HIV-2-specific CD8⁺ T-cells display an early or young differentiated phenotype, potentially reflecting their potential for T-cell renewal. They also harbor particularly potent effector functions, highlighted by their exceptional capacity to suppress the virus in autologous CD4⁺ T-cells. These CD8⁺ T-cell characteristics are similar to those observed in HIC1 in many aspects, and are likely to be part of the cause or consequence of effective control over the virus. This robust lymphopoietic potential is not preserved or restored in the CD8⁺ T-cells of HIV-1 infected patients undergoing antiretroviral treatment even when initiated during primary HIV-1 infection (41), which argues more in favor of causality.

Although overall, strong CD8⁺ T cell-mediated HIV-2 suppression is observed in HIC2 patients, some heterogeneity, as in HIC1, is observed in these patients (39, 49-51). The

absent or low CD8⁺ T cell-mediated HIV-2 suppression in four patients may be due to several factors. First, we cannot exclude the possibility that strong CD8⁺ T cell viral suppression was not elicited in these individuals due to other immunodominant epitopes that were not encoded by the HIV-2 SBL virus strain used in our experiments. However, we have previously shown that suppressive CD8 T-cell activity in HIV-1 controllers remained low in some donors even when autologous virus was used (39). Alternatively, the absence of suppressive activity may be a consequence of the stringent control of viremia, resulting in the contraction of the HIV-2 specific CD8⁺ T cell response to a small pool of high quality memory cells that fall below the detection threshold of our assay (49, 52, 53). Moreover, we were only able to analyse the CD8⁺ T cell response in blood, and would not be able to measure more robust responses that were active locally in patient tissues (54). Finally, other mechanisms such as innate or humoral responses, or infection with unfit virus, may exert a greater role than the activity of CD8⁺ T cells in controlling viremia in these patients.

The effective suppression of viral replication in HIC2 will prevent the development of chronic immune activation and further damage to the lymphopoietic system, thus allowing the maintenance of an effective CD8⁺ T-cell immune response which provides long-term control of the virus. This equilibrium between HIV-2 and host immunity may be referred to as a virtuous cycle, in contrast to the vicious cycle in HIV-1 infection, where apart from exceptional HIC1 cases, poorly controlled viral replication results in elevated chronic immune activation. The intensity of prolonged HIV-1 replication contributes to a process of premature immune aging that exerts its toll and further weakens cellular immunity in infected individuals.

We do not yet have a complete picture to explain the benign virtuous cycle of HIV-2 infection; whilst many epidemiological studies have found an association between specific HLA class I alleles and HIV-1 disease outcome, i.e. rapid or delayed progression to AIDS (11), the role of HLA polymorphism in HIV-2 is still unclear. Evidence exists that HLA-B*35 (55) and B*1503 (56) alleles are associated with HIV-2 disease progression; nevertheless several individuals bore HLA-B*35 alleles in our study, yet controlled their infection. Similarly, in the context of HIV-1 infection, HLA class I homozygosity is associated with rapid progression to AIDS (57, 58); however most of the HIC2 in our study are homozygotes. Interestingly, no HLA allele associated with control of infection has been found in HIV-2 infection. It was suggested by Yindom et al. (56) that this might be due to the fact that HIV-2 infection is easier to control compared to HIV-1, and therefore that less stringent requirements for control may not have led to the selection of “protective” epitopes that are restricted by specific HLA molecules during HIV-2 infection. Additionally, unlike the partial control exerted by CD8⁺ T cells in HIV-1 infection (59), the robust CD8⁺ T cell control of HIV-2 could limit the emergence of escape variants, and therefore abrogate the requirement for HLA Class I molecules that possess sufficient flexibility to adapt to new variants.

While still hypothetical at this stage, a greater sensitivity of HIV-2, compared with HIV-1, to intracellular restriction factors may play a crucial role in constraining the initial replication of HIV-2 in CD4⁺ T-cells. It has been shown that HIV-2 is more susceptible to restriction by tripartite motif protein isoform 5 alpha (TRIM5a) than HIV-1 (60). Subsequently, it was found that tetherin interacts differentially with the two viruses depending on whether it is counteracted by Vpu or Env, as is the case in HIV-1 and HIV-2 respectively (61, 62). This difference might reflect a different sensitivity of HIV-1 and HIV-2

to tetherin control at the cell membrane. cART-naïve HIV-2 infected patients from the ANRS CO5 HIV-2 Cohort also demonstrated high level of APOBEC3F/G editing activity in a previous study (63); however, immunovirological parameters were not found to be associated with the latter.

Finally, it has been proposed that the presence of the HIV-2/SIVsmm-specific Vpx, an accessory protein that inhibits the action of SAMHD1, enhances the sensing of HIV-2 by dendritic cells, which may promote the induction of more potent CD8⁺ T-cell responses (64). This requires further investigation, as other groups have reported that dendritic cells are refractory to HIV-2 infection, which could in turn, restrict HIV-2 replication (65, 66). Overall, an enhanced sensitivity of HIV-2 to host restriction factors may be crucial in limiting its replication initially, preventing further damage to the host immune system, providing the necessary window of opportunity for the subsequent induction and enactment of an effective T-cell response that establishes and maintains control of the virus. Once activated, this timely bottleneck would prevent the over-exertion and subsequent exhaustion of immune resources - usually related to the maintenance of the CD4 T-cell pool and the consumption of HIV-specific immune cells – together with the presence of the associated hyper inflammatory status often seen in HIV-1 infection; ultimately preserving the immune functions of the infected person. Combined together, this synergy in innate and adaptive factors may provide the necessary conditions for to establish the HIV-2 virtuous circle, and therefore exemplify a holistic mechanism for natural control of the virus.

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Footnotes

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2,3. These authors contributed equally to this work

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5. Abbreviations:

APOBEC 3F/G: Apolipoprotein B mRNA Editing Enzyme Catalytic Subunit 3F/G; *cART*: combination Antiretroviral Therapy; *HIC1/2*: HIV-1/2 Controller; *HPC*: Hematopoietic Progenitor Cell; *IQR*: Interquartile Range; *SAMHD1*: SAM domain and HD domain-containing protein 1; *sjTREC*: signal joint T-cell Receptor Excision Circle; *TRIM5α*: Tripartite Interaction Motif 5a.

Figure Legends

Figure 1. Lymphopoietic capacity of HIV-2 infected individuals. (a) Representative example of CD34 and lineage staining to identify HPC within total PBMCs from a HIV-2 infected patient (left panel) and absolute counts (right panel) of CD34⁺ Lin⁻ cells in middle aged healthy adults (HIV neg), treatment naïve viremic HIV-1 infected patients with high (HIV-1 CD4>500 CD4⁺ T-cells/ μ l) or low (HIV-1 CD4<200 CD4⁺ T-cells/ μ l) CD4⁺ T cell count, HIV-1 controllers (HIC1) and HIV-2 controllers (HIC2). (b) Representative example of CD117 and CD45RA staining on CD34⁺ enriched cells to identify circulating lymphoid precursors in a HIV-2 infected patient (left panel) and absolute counts (right panel) of CD117⁻ CD45RA⁺ CD34⁺ cells in middle aged healthy adults (HIV neg), treatment naïve viremic HIV-1 infected patients with high (HIV-1 CD4>500 CD4⁺ T-cells/ μ l) or low (HIV-1 CD4<200 CD4⁺ T-cells/ μ l) CD4⁺ T cell count, HIV-1 controllers (HIC1) and HIV-2 controllers (HIC2). (c) Representative example of CCR7 and CD45RA staining on CD8⁺ T-cells to identify naïve cells in a HIV-2 infected patient (left panel) and frequency (right panel) of naïve cells in middle aged healthy adults (HIV neg), treatment naïve viremic HIV-1 infected patients with high (HIV-1 CD4>500 CD4⁺ T-cells/ μ l) or low (HIV-1 CD4<200 CD4⁺ T-cells/ μ l), HIV-1 controllers (HIC1) and HIV-2 controllers (HIC2). (d) Correlation between sj TREC levels (sjTREC/mL) and naïve CD8⁺ T-cell counts in HIV-2 controllers. (e) Correlation between the counts of recent thymic emigrants and naïve CD8⁺ T-cells in HIV-2 controllers. The Mann-Whitney test was used for comparing groups. The Spearman's rank test was used to determine correlations. Bars indicate the median.

Figure 2. Characterization of p27-specific CD8⁺ T-cells in HIV-2 infected individuals. (a) Representative example of IFN γ secretion in CD8⁺ T-cells from a HIV-2 infected patient

unstimulated or upon stimulation with p27 overlapping peptides (left panel) and frequencies of IFN γ ⁺ CD8⁺ T-cells in HIV-1 controllers (HIC1) and HIV-2 controllers (HIC2) upon stimulation with HIV-1 and HIV-2 peptides respectively (right panel). **(b)** Representative stainings of HLA-B*5301 restricted p27 TPYDINQML (TL9) (left panel) or HLA-B*1401 restricted p27 DRFYKSLRA (DA9) (right panel) specific CD8⁺ T-cells from HIV-2 infected patients. **(c)** Representative stainings for the expression of the cell surface markers CD27, CD28, CD45RA, CCR7, CD127 and intracellular granzyme B in TL9-specific CD8⁺ T-cells. **(d)** Comparative expression of the cell surface markers CD27, CD28, CD45RA, CCR7, CD127 and intracellular granzyme B between B27 KK10-specific CD8⁺ T-cells from HIV-1 infected patients (open diamonds) and B53 TL9 or B14 DA9-specific CD8⁺ T-cells from HIV-2 infected patients (full circles). The Mann-Whitney test was used for comparing groups. Bars indicate the median.

Figure 3. *Ex vivo* viral suppressive capacity of HIV-2 infected individuals CD8⁺ T-cells. **(a)** Examples of p27 concentration in supernatant of CD4⁺ T-cells isolated from an HIV-2 controller (top) or an healthy donor (bottom), alone (red) or co-cultured with autologous CD8⁺ T-cells (blue) at different days post infection. Data are represented as Means and Standard Deviations. **(b)** Log p27 decrease (p27 concentration in supernatant of infected CD4⁺ T-cells alone divided by p27 concentration in supernatant of infected CD4⁺ T-cells co-cultured in presence of autologous CD8 T-cells) in HIV-1 (open diamonds) or HIV-2 controllers (black circles). Bars indicate the median. **(c)** p27 concentration in supernatant of CD4⁺ T-cells isolated from 3 different HIV-2-infected individuals, alone (red columns) or co-cultured with autologous CD8 T-cells (blue columns) at time of inclusion (Month 0, M0) or 12 months later (M12). Histograms are represented as Means and Standard Deviations.

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717 **Figure 4. HIV-2 viral suppressive function and markers of disease progression. (a)** p27
718 concentrations in supernatant of CD4⁺ T-cells isolated from HIV-2 infected individuals with
719 rapidly decreasing CD4⁺ T-cells during at least 3 years (92.5 cells/mm³/year (left) and 215.7
720 cells/mm³/year (right)), alone (red) or co-cultured with autologous CD8 T-cells (blue) at
721 different days post infection. Data are represented as Means and Standard Deviations. **(b)**
722 Correlation of CD4⁺ T-cell counts (cells/mm³) and log p27 decrease (p27 concentration in
723 supernatant of infected CD4⁺ T-cells alone divided by p27 concentration in supernatant of
724 infected CD4⁺ T-cells co-cultured in presence of autologous CD8 T-cells) in HIV-2-infected
725 individuals. The Spearman's rank test was used to determine correlations.

726

Fig. 1

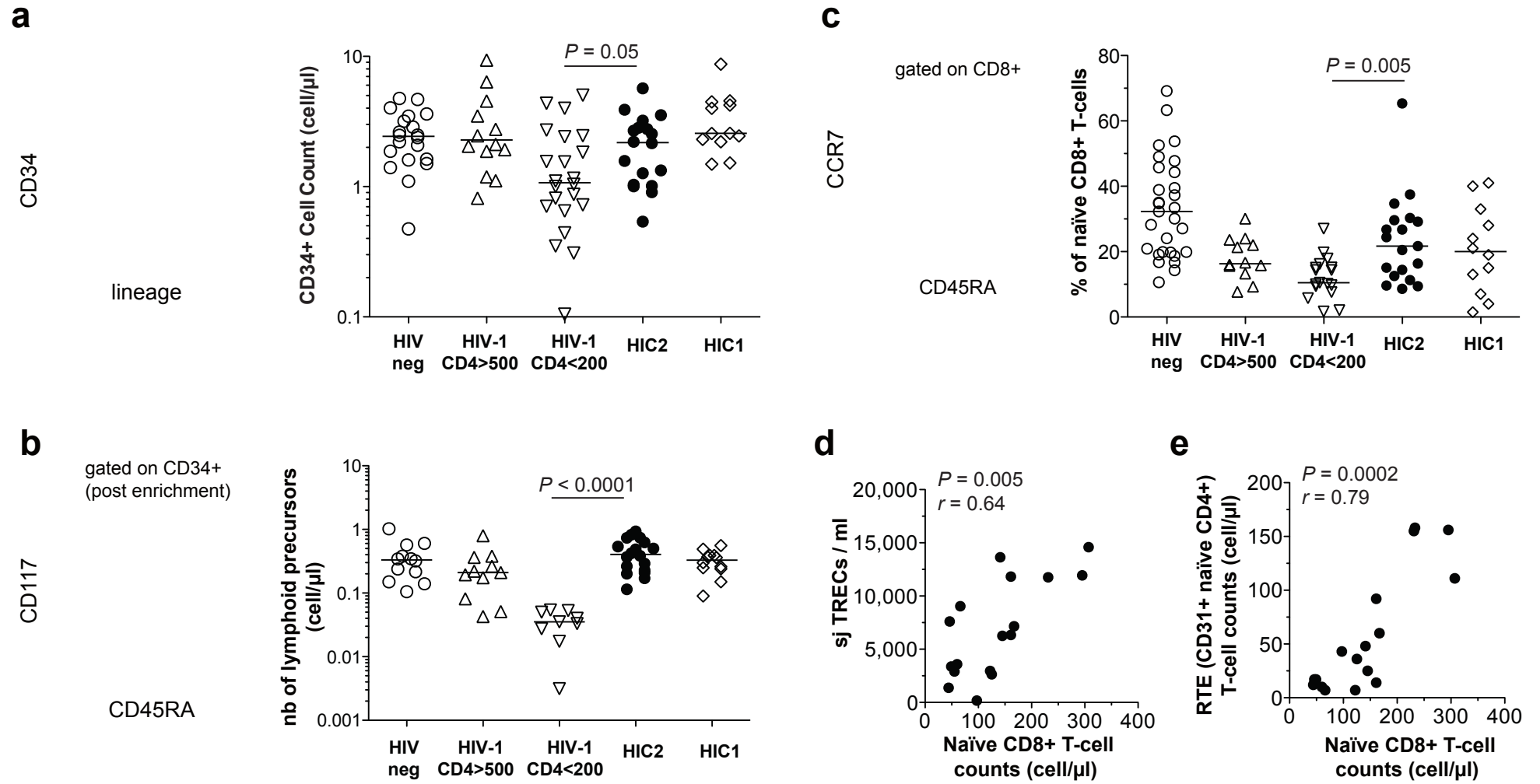


Fig. 2

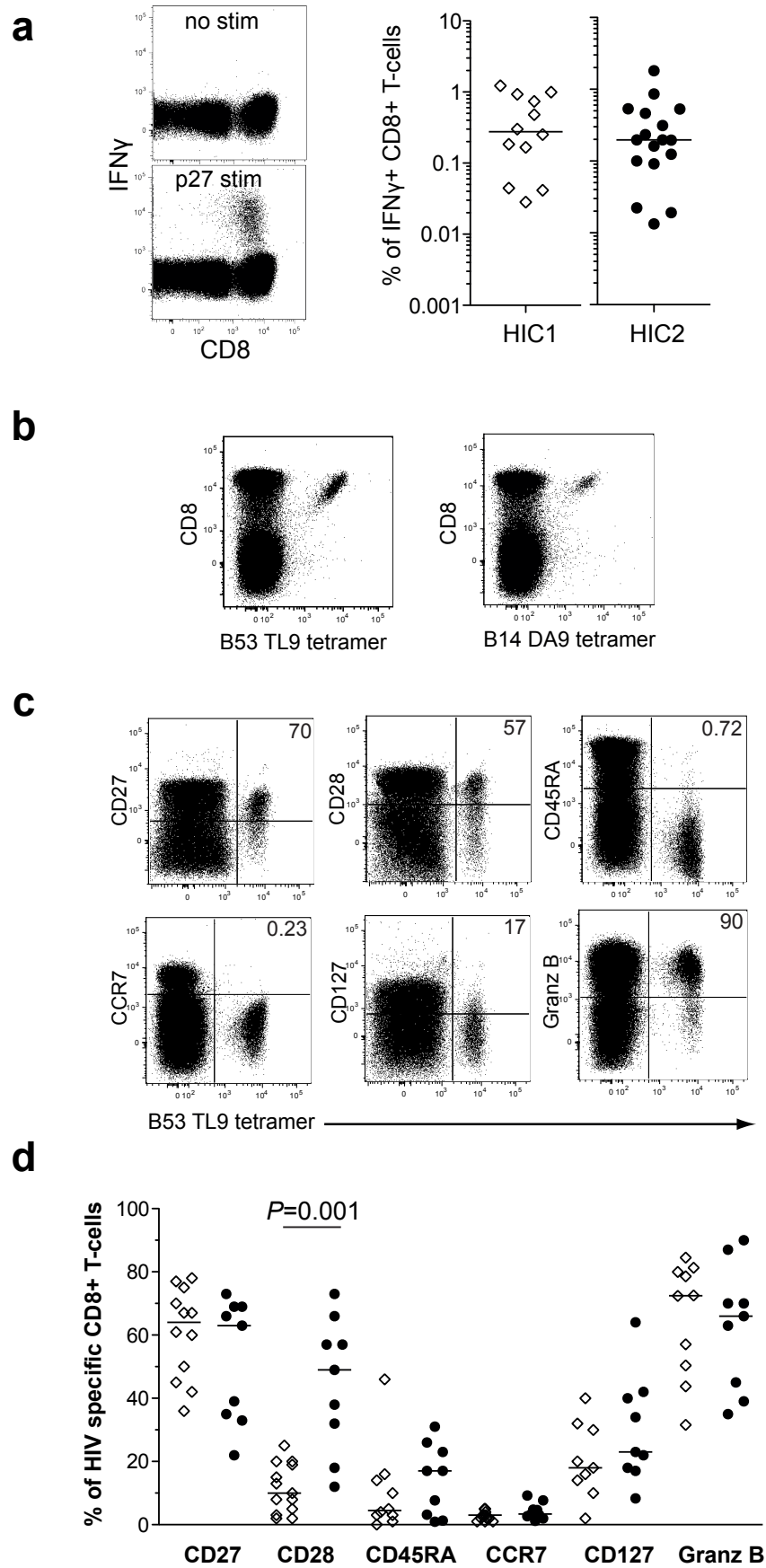


Fig. 3

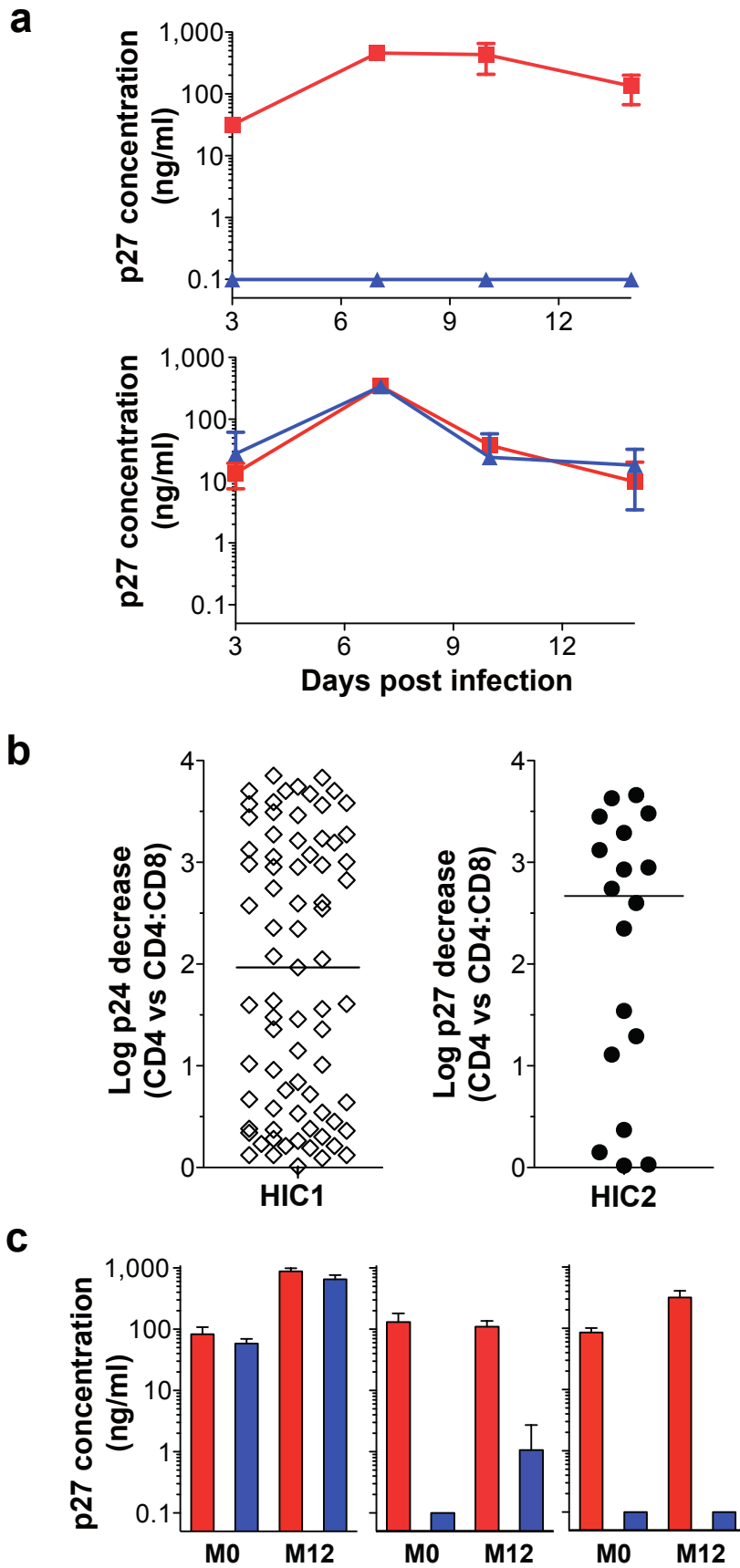
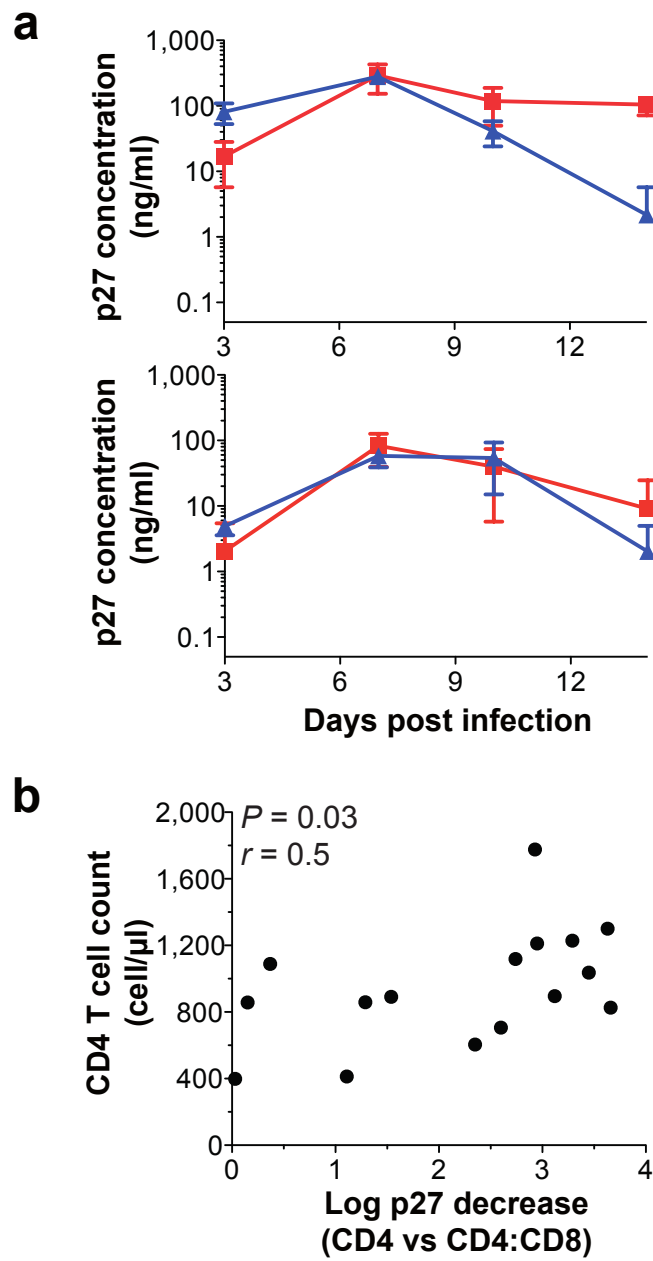


Fig. 4



1 **Table I. Clinical attributes of the HIV-2-infected patients studied**

ID	Gender	Age (years)	CD4 count (cells/ μ l)	Time since diagnosis (years)	Viral load (copies/mL)	HLA A	HLA B	HLA C	Country of Birth
012-006	M	44	707	20.5	<40	03/23	35/53	04/04	Ivory Coast
012-009	F	54	1,118	27.3	<100	03/74	14/15	07/08	Guinea Conakry
012-045	F	43	858	22.7	<40	1/33	15/35	04/14	France
012-073	M	52	891	11.3	<100	02/03	49/57	07/18	Ivory Coast
012-084	F	50	1,776	8.8	<40	68/68	07/52	-	Ghana
012-088	M	70	502	7.4	117	02/02	27/53	02/04	Senegal
012-101	F	34	1,212	12.5	<40	03/26	58/58	03/07	The Gambia
013-035	F	48	859	9.2	<40	34/34	15/53	02/04	Guinea Conakry
013-037	M	59	1,300	8.8	<40	01/29	44/57	06/16	Colombia
013-049	F	52	604	25.6	<40	02/23	15/52	02/16	Guinea Conakry
019-010	F	40	1,170	15.4	-	33/68	53/53	04/04	Ivory Coast
023-008	F	41	-	5.0	<40	34/34	7/53	04/07	Ivory Coast
028-012	F	28	827	10.2	<40	-	18/78	05/16	Guinea Conakry
028-016	F	49	895	11.3	<40	02/68	15/51	14/16	Ivory Coast
036-018	F	39	1,036	12.7	<100	03/03	35/53	04/04	Ivory Coast
036-019	M	53	1,228	17.6	<40	23/23	07/14	07/08	Guinea Bissau
045-007	F	50	-	16.2	40	33/33	53/53	04/04	Ivory Coast
051-007	M	48	399	14.4	<40	02/29	39/44	07/16	France
075-001	M	52	413	18.2	54	24/25	35/44	05/12	France
082-005	M	57	1,090	23.0	<100	23/34	53/53	04/04	Ivory Coast

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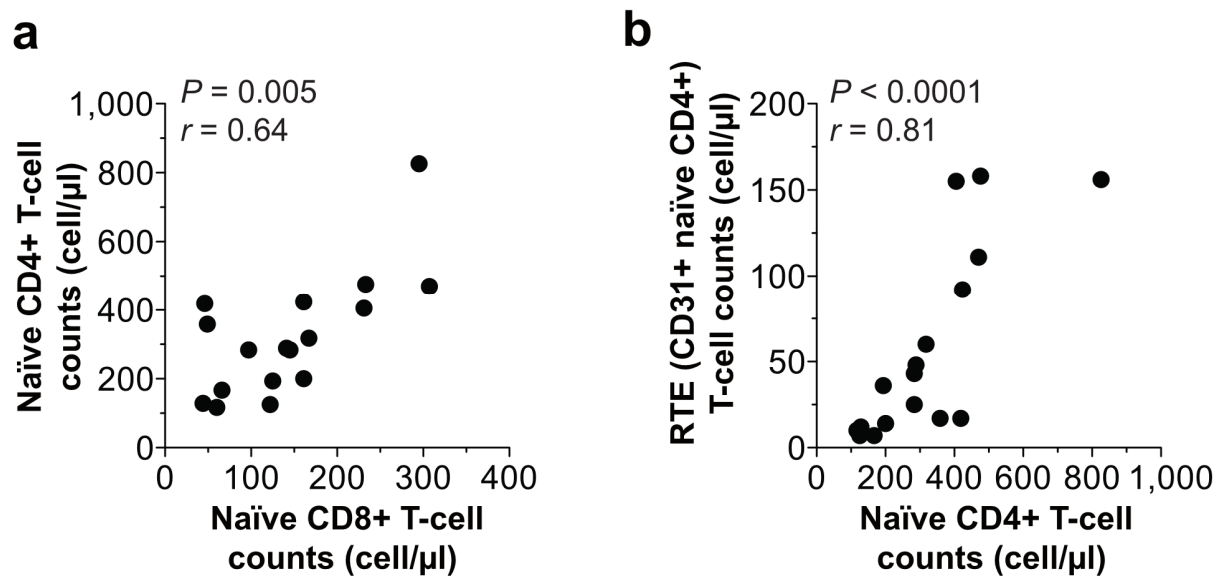
1 **Table II. Summary of clinical attributes of the patients studied**

Group	Gender (%female)	Age (years)	CD4 count (cells/ μ L)	Viral load (copies/mL)
HIV1-negative	51%	39 [33-47]	860 [610-1,110]	NA
HIV1+ CD4<200	30%	40 [32-48]	82 [42-136]	127,330 [3,040-543,000]
HIV1+ CD4>500	11%	39 [31-48]	690 [560-910]	16,540 [800-6,300]
HIV1+-controllers	53%	48 [42-51]	751 [519-953]	<40 [<40-57]
HIV2+-controllers	60%	49 [42-52]	893 [707-1,170]	<40 [<40-88.5]

2 Values are expressed as: median [interquartile range]

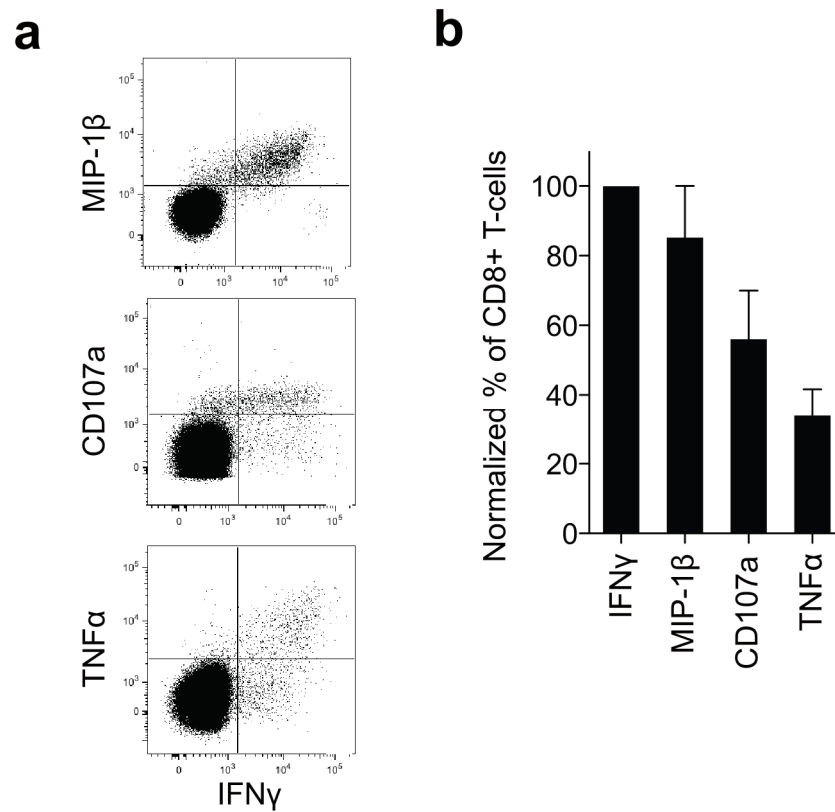
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Supplementary Figure 1. Naïve T-cell count levels in HIV-2 infected individuals.



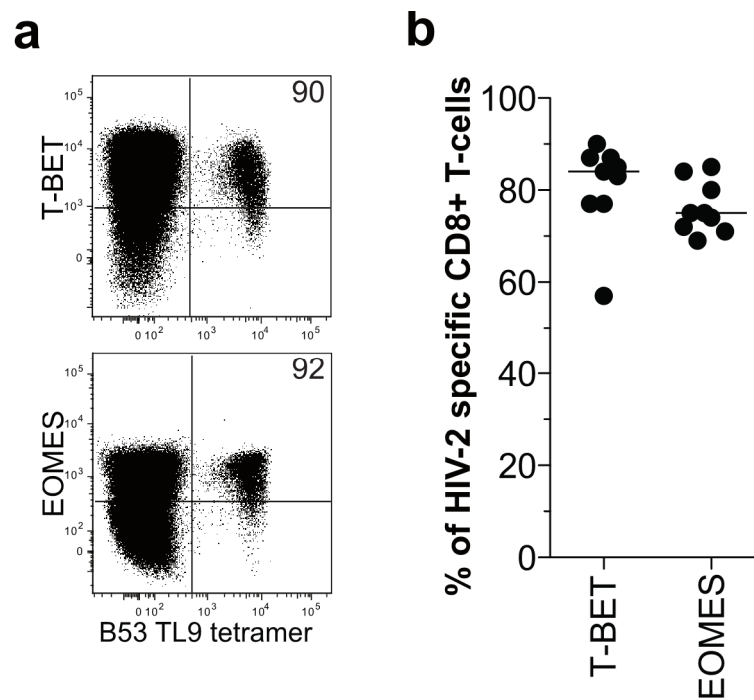
(a) Correlation between naïve CD8⁺ and naïve CD4⁺ T-cell counts in HIV-2 controllers. (b) Correlation between the counts of recent thymic emigrants (RTE) and naïve CD4⁺ T-cells in HIV-2 controllers. The Spearman's rank test was used to determine correlations.

Supplementary Figure 2. Polyfunctional potential of HIV-2 p27-specific CD8⁺ T-cells.



(a) Representative examples of MIP-1β, CD107a, TNFα and IFNγ secretion in CD8⁺ T-cells from a HIV-2 infected patient upon stimulation with p27 overlapping peptides. (b) Frequencies of CD8⁺ T-cells secreting IFNγ, MIP-1β, CD107a or TNFα in HIV-2 infected patients upon stimulation with p27 overlapping peptides respectively. Data are normalized mean percentages from nine patients

Supplementary Figure 3. Expression of T-BET and EOMES in HIV-2 p27-specific CD8⁺ T-cells.



(a) Representative stainings for the intracellular expression of the transcription factors T-BET and EOMES in B53-TL9-specific CD8⁺ T-cells. **(b)** Expression of the transcription factors T-BET and EOMES in B53 TL9 or B14 DA9-specific CD8⁺ T-cells from HIV-2 infected patients.